

The *Caenorhabditis elegans* p120 catenin homologue, JAC-1, modulates cadherin–catenin function during epidermal morphogenesis

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The cadherin–catenin complex is essential for tissue morphogenesis during animal development. In cultured mammalian cells, p120 catenin (p120ctn) is an important regulator of cadherin–catenin complex function. However, information on the role of p120ctn family members in cadherin-dependent events in vivo is limited. We have examined the role of the single *Caenorhabditis elegans* p120ctn homologue JAC-1 (juxtamembrane domain [JMD]–associated catenin) during epidermal morphogenesis. Similar to other p120ctn family members, JAC-1 binds the JMD of the classical cadherin HMR-1, and GFP-tagged JAC-1 localizes to adherens junctions in an HMR-1–dependent manner. Surprisingly, depleting JAC-1 expression using

RNA interference (RNAi) does not result in any obvious defects in embryonic or postembryonic development. However, *jac-1(RNAi)* does increase the severity and penetrance of morphogenetic defects caused by a hypomorphic mutation in the *hmp-1/α-catenin* gene. In these *hmp-1* mutants, *jac-1* depletion causes failure of the embryo to elongate into a worm-like shape, a process that involves contraction of the epidermis. Associated with failed elongation is the detachment of actin bundles from epidermal adherens junctions and failure to maintain cadherin at adherens junctions. These results suggest that JAC-1 acts as a positive modulator of cadherin function in *C. elegans*.

Introduction

Proper development requires dynamic regulation of cadherin-mediated adhesions (Tepass et al., 2000). Cadherins are calcium-dependent homodimeric cell–cell adhesion receptors that are key components of the apical adherens junctions common to all epithelial cells. The intracellular tails of cadherin molecules are linked to the actin cytoskeleton by the cytoplasmic proteins β- and α-catenin. β-Catenin binds directly to the COOH terminus of cadherin and to α-catenin. α-Catenin can bind F-actin and several proteins with actin binding, bundling, and remodelling activities (Vasioukhin and Fuchs, 2001) and thereby serves to link the cadherin adhesion interface to the actin cytoskeleton.

Another cadherin-binding protein, p120 catenin (p120ctn),* was originally identified as a potent Src substrate (Reynolds et al., 1994) and subsequently shown to have an important, yet complex, role in regulating cadherin function. p120ctn binds the membrane-proximal juxtamembrane domain (JMD) of cadherin, a site that has been implicated in the regulation of cadherin activity (for review see Anastasiadis and Reynolds, 2000). p120ctn has been proposed to positively regulate cadherin-mediated adhesion by mediating lateral clustering of cadherin, thereby promoting the transition from weak to strong adhesion (Yap et al., 1998; Thoreson et al., 2000), and through stabilizing cadherin molecules at adherens junctions (Ireton et al., 2002). However, there is also evidence that p120ctn may negatively regulate cadherin-mediated adhesion, which may depend on its phosphorylation state (for review see Anastasiadis and Reynolds, 2000). In addition, p120ctn can also regulate the activity of Rho family GTPases (for review see Anastasiadis and Reynolds, 2001), which are key regulators of actin dynamics and cell adhesion. Whether p120ctn positively or negatively regulates cadherin-mediated adhesion appears to be dependent on cell type and the biochemical state of the cell.

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*Abbreviations used in this paper: Armadillo, Arm; CFB, circumferential actin filament bundle; Fn3, fibronectin type III; JMD, juxtamembrane domain; p120 catenin, p120ctn; RNAi, RNA interference.

Key words: adherens junctions; p120ctn; α-catenin; embryo development; actin filaments

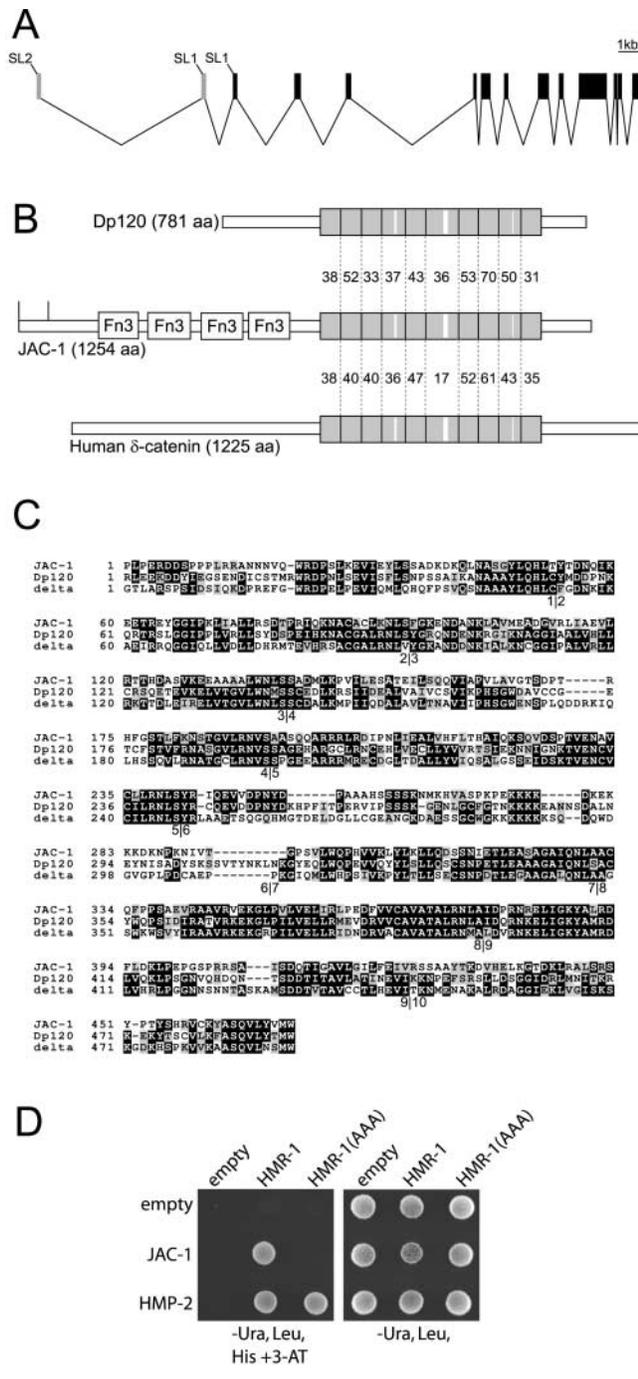


Figure 1. *jac-1* encodes a *C. elegans* p120ctn homologue that interacts specifically with the JMD of HMR-1. (A) Structure of the *jac-1* gene. Exons are represented by shaded boxes; two putative alternative exons are lightly shaded. The spliced-leader sequences found at the start of each alternative *jac-1* transcript are indicated. (B) Schematic structure of JAC-1 compared with Dp120ctn and human δ -catenin (GenBank/EMBL/DDJB accession nos. AAF33245 and Q9UQB3, respectively). Fn3 domains and Arm repeats are represented as open and shaded boxes, respectively. Interruptions in Arm repeats 4, 6, and 9 are indicated by unshaded regions. The numbers indicate the percent amino acid identity shared between corresponding Arm repeats. Predicted alternative start codons for JAC-1 are indicated by vertical lines. (C) ClustalW alignment of the Arm repeat regions (as defined by Anastasiadis and Reynolds, 2000) of JAC-1, Dp120, and δ -catenin (beginning at amino acids 594, 201, and 527, respectively). Black countershading indicates identical

A small number of studies have examined the role of p120ctns during morphogenesis. Overexpression of murine p120ctn in *Xenopus laevis* causes morphogenetic defects consistent with its proposed role in regulating cadherin-dependent cell adhesion (Paulson et al., 1999). Recent studies in *Drosophila* indicate that neither the single p120ctn homologue (Dp120ctn) nor the JMD of DE-cadherin is essential for development (Myster et al., 2003; Pacquelet et al., 2003). However, a null mutation in Dp120ctn enhances the severity of DE-cadherin and Armadillo (Arm)/ β -catenin loss-of-function phenotypes, implicating Dp120ctn in the regulation of cadherin function (Myster et al., 2003). Furthermore, overexpression of Dp120ctn results in enhancement of Rho1 loss-of-function phenotypes, suggesting that Dp120ctn may affect the function or levels of Rho1 (Magie et al., 2002).

To gain more insight into the role played by p120ctns during animal development, we have characterized p120ctn function during epidermal morphogenesis in *C. elegans*. The epidermis is responsible for the elongation of the initially ellipsoidal embryo along its anterior–posterior axis, through the coordinated circumferential contraction of individual epidermal cells (Priess and Hirsh, 1986). The *C. elegans* cadherin–catenin complex, consisting of a classical cadherin (HMR-1), a β -catenin (HMP-2), and an α -catenin (HMP-1), is essential for epidermal morphogenesis (Costa et al., 1998; Raich et al., 1999). We show here that the single *C. elegans* p120ctn homologue positively modulates cadherin function during embryonic elongation by promoting proper association between the actin cytoskeleton and the cadherin–catenin complex.

Results and discussion

We, and others (Natarajan et al., 2001), have identified a single predicted gene, Y105C5B.21, that is capable of encoding a p120ctn homologue (Fig. 1). Based on the sequence similarity and functional analysis described below, we have given this gene the designation *jac-1* (JMD-associated catenin). *jac-1* is predicted to encode two isoforms that differ only in their extreme NH₂ termini, and we will thus not distinguish between them in the following discussion. JAC-1 contains the arrangement of 10 Arm repeats characteristic of p120ctns (Fig. 1, B and C; Anastasiadis and Reynolds, 2000). As found in comparisons between Arm/ β -catenin homologues (Peifer et al., 1994), each JAC-1 Arm repeat shows higher similarity to the corresponding repeat in Dp120 and δ -catenin (the mammalian p120ctn to which it is most similar) than it does to the other JAC-1 Arm repeats (Fig. 1 B), suggesting functional conservation of each repeat.

The NH₂ termini of mammalian p120ctns include motifs implicated in protein–protein interactions (Anastasiadis and Reynolds, 2000) and regulation via tyrosine phosphoryla-

residues; gray, similarity. The borders of each Arm repeat are indicated by vertical lines. (D) JAC-1 interacts specifically with the JMD of HMR-1 in the yeast two-hybrid system. Growth on minus Ura, Leu, His media containing 1 mM 3-AT indicates interaction between the activation domain fusion proteins (JAC-1 and HMP-2) and the binding domain fusion proteins (HMR-1).

tion (Mariner et al., 2001). We could not identify equivalent motifs in the region of JAC-1 NH₂ terminal to the Arm repeats. Instead, this region contains four fibronectin type III (Fn3) domains that show closest similarity to Fn3 domains found in intracellular muscle proteins, such as titin, twitchin, and projectin (Kenny et al., 1999). The functional significance of these domains is unclear, but they are also found in the *C. briggsae* JAC-1 homologue.

The COOH terminus of JAC-1 ends in a putative type I PDZ (PSD-95/Discs-large/ZO-1) domain-binding motif (DWSL), also found in several vertebrate p120ctns (Anastasiadis and Reynolds, 2000) but absent from p120ctn itself and Dp120ctn. In δ -catenin and p0071, this motif interacts with the PDZ domain of the LAP (leucine-rich repeats and PDZ domains) family protein Erbin (Jaulin-Bastard et al., 2002; Laura et al., 2002).

Previous work has shown that the Arm repeat region of p120ctns directly interacts with the JMD of cadherins (Anastasiadis and Reynolds, 2000). Using the yeast two-hybrid system, we determined that a fragment of JAC-1 containing the Arm repeat region and COOH terminus interacts with the intracellular domain of HMR-1 (Fig. 1 D). To confirm that JAC-1 specifically interacts with the JMD of HMR-1, we created a mutant version of the HMR-1-Gal4 DNA-binding domain construct in which the highly conserved EGGGE motif was changed to EAAAE. The same amino acid substitutions block the interaction between mammalian E-cadherin and p120ctn (Thoreson et al., 2000). Mutation of these residues abolished the interaction between JAC-1 and HMR-1, however its binding to HMP-2/ β -catenin was unaffected, indicating that mutation of the JMD specifically disrupts binding of JAC-1. These experiments demonstrate that JAC-1 interacts with the HMR-1 JMD, as expected of a bona fide p120ctn.

To gain insight into the physiological function of JAC-1, its expression and subcellular localization were examined. In situ hybridization demonstrates that *jac-1* mRNA is expressed in the early epidermis that lies on the dorsal posterior side of the embryo (Fig. 2 A). No available antibodies cross-react with JAC-1, so a *jac-1-gfp* construct driven by an epithelial promoter was used to visualize JAC-1 localization. JAC-1-GFP is present at epidermal cell borders throughout morphogenesis. Epidermal morphogenesis in *C. elegans* involves three steps: dorsal intercalation, ventral enclosure, and elongation (for review see Simske and Hardin, 2001). JAC-1-GFP is present at the borders of intercalating cells in the dorsal epidermis (Fig. 2 B, a). During ventral enclosure, JAC-1-GFP concentrates at the lateral edges of cells near the leading edge (Fig. 2 B, d and e) and is observed at sites of cell-cell contact at the ventral midline (Fig. 2 B, f). JAC-1-GFP continues to localize to epidermal cell borders during elongation (Fig. 2 B, b and c).

To determine if JAC-1 localizes specifically to adherens junctions, embryos expressing JAC-1-GFP were stained for HMR-1. We found that JAC-1-GFP and HMR-1 precisely colocalize in the epidermis (Fig. 3, A–C). In contrast, JAC-1-GFP localizes apically with respect to AJM-1 (Fig. 3, D–F), a junctional component that localizes basally to HMR-1 (Koppen et al., 2001). To determine if the membrane localization of JAC-1 requires HMR-1, JAC-1-GFP localization was ob-

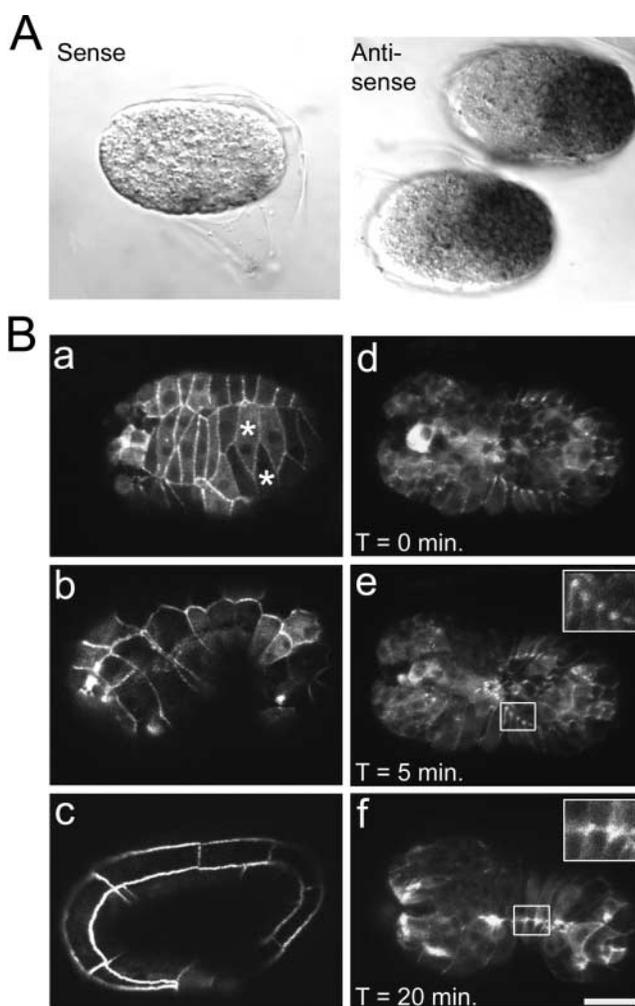


Figure 2. *jac-1* is expressed in the early epidermis and JAC-1-GFP localizes to epidermal cell borders during morphogenesis.

(A) Representative images from in situ hybridization with a control *jac-1* sense probe (left) and *jac-1* antisense probe (right). (B) Confocal images of JAC-1-GFP expression. (a) Dorsal view showing JAC-1-GFP localization during dorsal intercalation (two interdigitating cells are marked with asterisks). (b and c) Lateral views showing JAC-1-GFP localization during elongation. (d–f) Time course of an embryo undergoing ventral enclosure. Bar, 10 μ m.

served in embryos homozygous for the *hmr-1(zu389)*-null mutation and in *hmr-1(RNAi)* embryos. In both cases, JAC-1-GFP failed to localize to cell junctions (Fig. 3, G and H; unpublished data). Together these data indicate that JAC-1 localizes to adherens junctions in a cadherin-dependent manner, as has previously been shown for vertebrate p120ctns.

As no *jac-1* mutants currently exist, the role of JAC-1 during epidermal morphogenesis was assessed through depleting its expression via RNA interference (RNAi). Depletion of *jac-1* function in wild-type animals did not produce any observable defects. We also examined the effect of *jac-1(RNAi)* on a mutant strain (NL2099) shown to be hypersensitive to RNAi (Simmer et al., 2002) and, similarly, did not detect a mutant phenotype. Semiquantitative RT-PCR analysis revealed that *jac-1* transcript levels were reduced \sim 15-fold (15.56 ± 2.30 SD) compared with controls (Fig. 4 A), indicating that significant reduction in *jac-1* expression

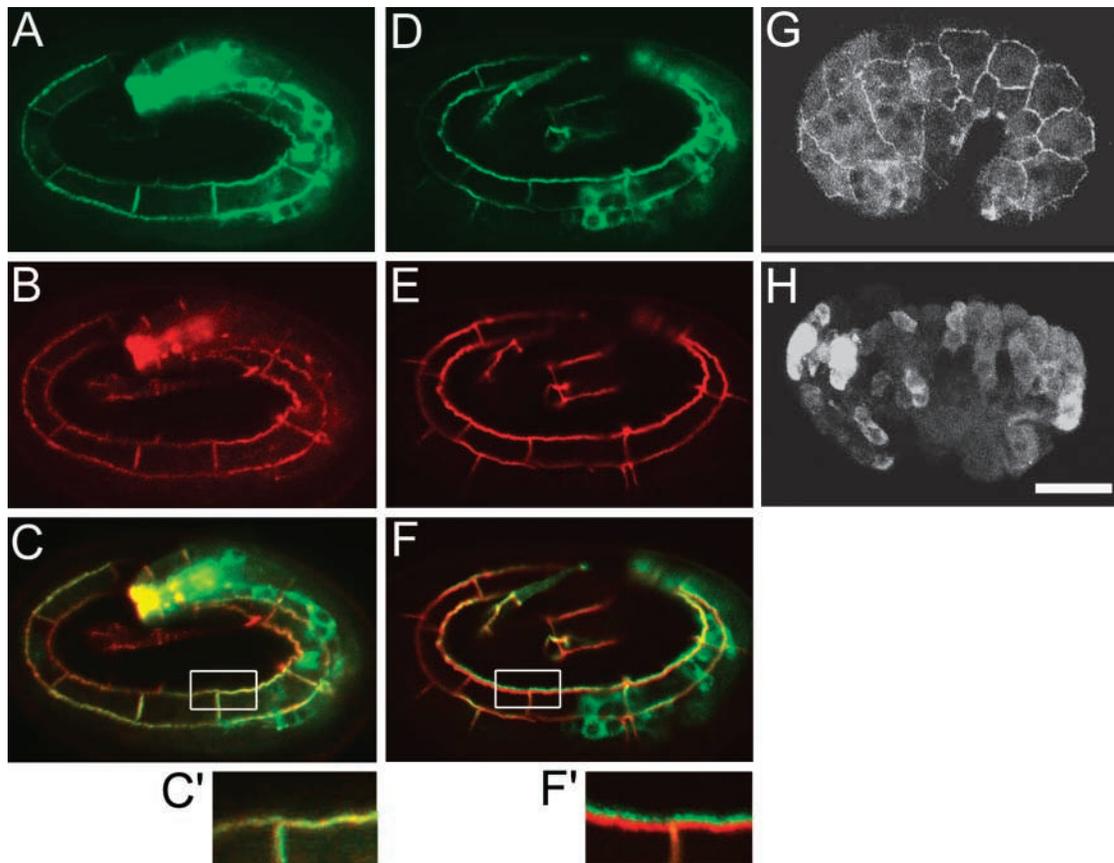


Figure 3. JAC-1-GFP localizes to adherens junctions in an HMR-1-dependent manner. (A–C) Confocal images showing a lateral view of an embryo expressing JAC-1-GFP (green) stained for HMR-1 (red). The merged image is shown in C, and the boxed region is enlarged 2.5 \times in C'. (D–F) Confocal images showing a lateral view of an embryo expressing JAC-1-GFP (green) stained for AJM-1 (red). The merged image is shown in F, and the boxed region is enlarged 2.5 \times in F'. (G and H) JAC-1-GFP expression in a wild-type (G) and *hmr-1(zu389)* embryo (H) of approximately the same age. Bar, 10 μ m.

has no observable phenotypic effect. In contrast, RNAi depletion of core cadherin–catenin components (HMR-1, HMP-1, or HMP-2) leads to fully penetrant embryonic lethality (Costa et al., 1998; Raich et al., 1999), suggesting that JAC-1 may not play an essential role in cadherin–catenin function. This is consistent with recent studies in *Drosophila* in which Dp120ctn-null embryos (Myster et al., 2003) and DE-cadherin mutants lacking the JMD (Pacquelet et al., 2003) were found to exhibit normal development.

To further investigate the function of JAC-1, we examined the effect of *jac-1(RNAi)* in animals with compromised cadherin–catenin function. We recently isolated a weak *hmp-1* hypomorphic mutation, *fe4*, which reduces, but does not completely abolish, cadherin–catenin function. *fe4* is a missense mutation resulting in a serine to phenylalanine substitution at amino acid 823, in the third vinculin homology domain of HMP-1. In other systems, this domain binds F-actin, vinculin, and ZO-1 (for review see Vasioukhin and Fuchs, 2001). Based on anti-HMP-1 immunostaining, the *fe4* mutation does not prevent HMP-1 localization to cell junctions (unpublished data). Approximately 42% of *hmp-1(fe4)* homozygotes fail to elongate properly, and arrest during late embryonic or early larval development with body shape defects. Those that reach adulthood are fertile with variable defects in body morphology.

jac-1(RNAi) increases the penetrance of the embryonic and early larval lethality of *hmp-1(fe4)* homozygotes from 42% ($\pm 12.2\%$ SD; $n = 1,118$) to 97% ($\pm 2.9\%$ SD; $n = 1,099$), indicating that JAC-1 has a role in modulating cadherin function. 4D Nomarski microscopy analysis (Fig. 4 B; see Videos 1–4, available at <http://www.jcb.org/cgi/content/full/jcb.200212136/DC1>) indicates that elongation fails in 89% of *hmp-1(fe4); jac-1(RNAi)* embryos ($n = 56$) (Fig. 4 B, d). In these embryos, development proceeds normally until the 1.25 fold stage, whereupon the embryos exhibit a large dorsal bulge, develop a dorsal flexure, and fail to elongate. These defects closely resemble the phenotypes previously described for a null allele of *hmp-1* (Costa et al., 1998). Such phenotypes were observed in only 10% of *hmp-1(fe4)* embryos examined ($n = 79$). These results suggest that JAC-1 positively modulates cadherin/catenin function during epidermal morphogenesis.

As elongation defects have been associated with defects in the actin cytoskeleton of the epidermis (for review see Chinsang and Chisholm, 2000; Simske and Hardin, 2001), we examined the effects of *jac-1(RNAi)* on actin structure. In wild-type embryos, the circumferential actin filament bundles (CFBs) that drive elongation are evenly distributed in parallel arrays throughout the epidermis (Fig. 5 A, a). *jac-1(RNAi)* does not result in any observable CFB defects (Fig.

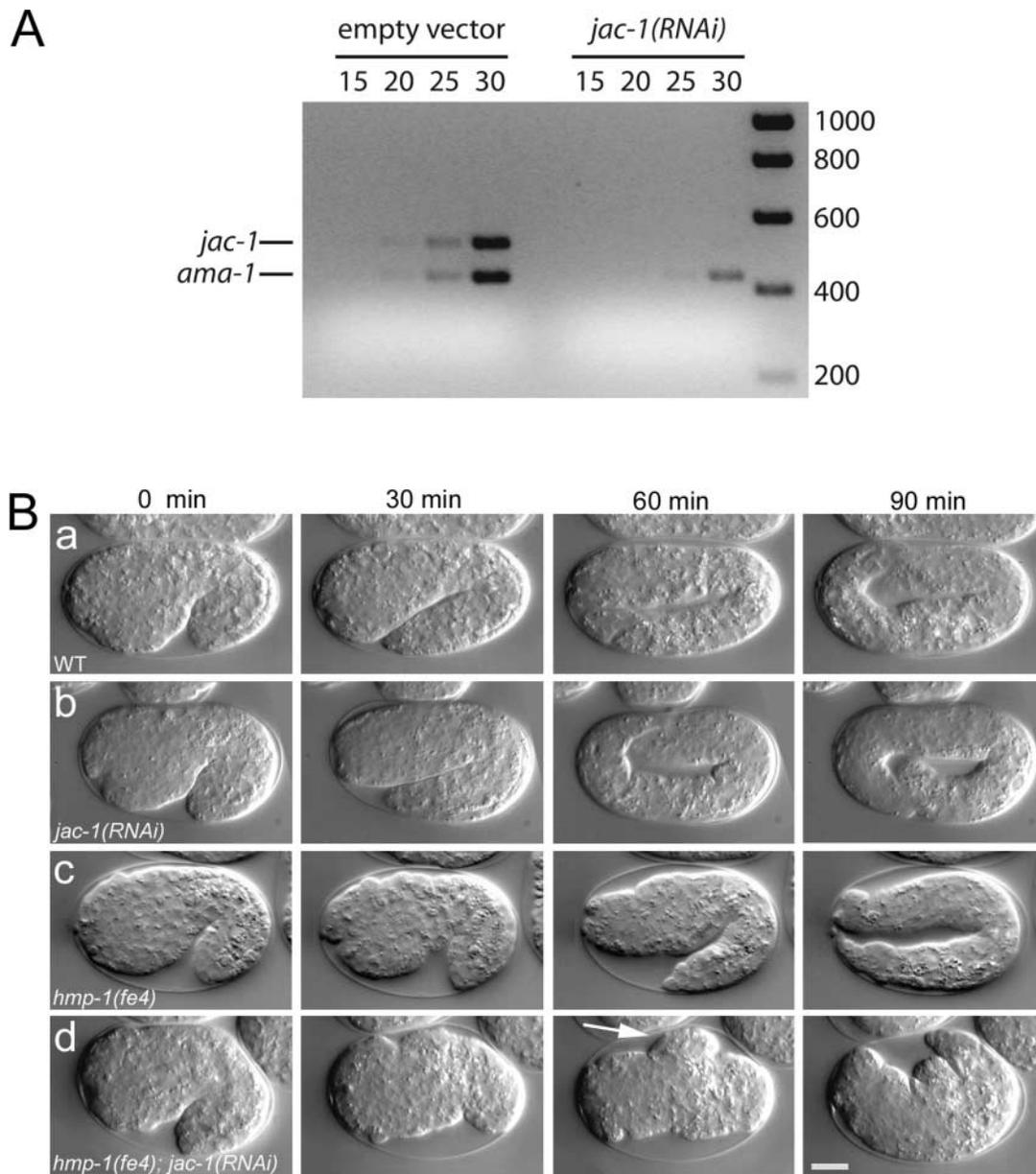


Figure 4. ***jac-1(RNAi)* enhances the elongation defects of *hmp-1(fe4)* mutants.** (A) Agarose gel electrophoresis of semiquantitative RT-PCR reactions derived from worms grown on HT115(DE3) bacteria carrying either the empty RNAi feeding vector or the *jac-1(RNAi)* feeding vector. Numbers on top indicate the number of amplification cycles. Numbers on the side indicate the base pair size of the marker DNAs. (B) Nomarski images at 30-min intervals of representative embryos undergoing elongation. Corresponding videos (Videos 1–4) are available at <http://www.jcb.org/cgi/content/full/jcb.200212136/DC1>. (a) Wild-type embryo. (b) *jac-1(RNAi)* embryo. (c) *hmp-1(fe4)* embryo. (d) *hmp-1(fe4); jac-1(RNAi)* embryo. Bar, 10 μ m.

5 A, b), consistent with our failure to detect morphogenetic defects with this treatment. *hmp-1(fe4)* embryos exhibit varying degrees of defects in CFBs, indicating that the *fe4* mutation weakens the linkage between cadherin complexes and actin. These embryos typically have several clusters of CFBs that are thicker than those observed in wild-type embryos, and these thick bundles typically correlate with pinches in the surface of the embryo, giving it a lumpy appearance (Fig. 5 A, c). In *hmp-1(fe4); jac-1(RNAi)* embryos, the CFBs are always severely disrupted (Fig. 5 A, d). Disorganized and abnormally thick actin filament bundles are observed, and CFBs often are detached from adherens junctions,

particularly in the dorsal epidermis. This phenotype resembles that exhibited by the most severe *hmp-1(fe4)* embryos (unpublished data) and by *hmp-1* nulls (Costa et al., 1998). The severe CFB defects implicate JAC-1 in the proper anchorage of CFBs at epidermal adherens junctions during elongation and are likely to account for the failed elongation of *hmp-1(fe4); jac-1(RNAi)* embryos.

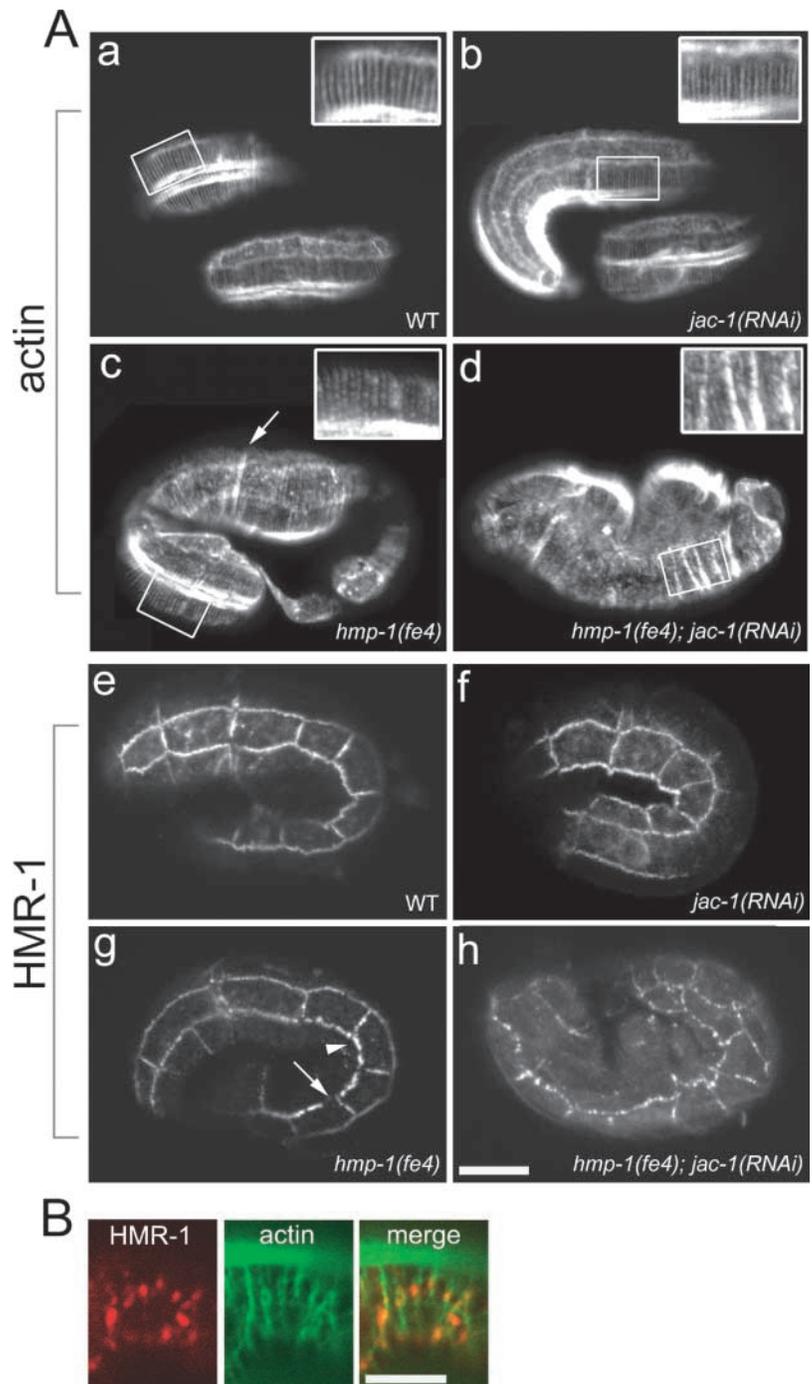
To determine if JAC-1 has a role in regulating cadherin distribution during epidermal morphogenesis, staining for HMR-1/cadherin was performed. Throughout epidermal morphogenesis, wild-type embryos exhibit continuous HMR-1 localization along epidermal adherens junctions

(Fig. 5 A, e). *jac-1(RNAi)* embryos do not exhibit any detectable alterations in HMR-1 distribution (Fig. 5 A, f). In *hmp-1(fe4)* homozygotes, a range of defects in HMR-1 distribution are evident during elongation. In most embryos, HMR-1 localizes normally to junctions, with occasional patches where HMR-1 is discontinuous (Fig. 5 A, g). *hmp-1(fe4); jac-1(RNAi)* embryos exhibit normal HMR-1 distribution until the start of elongation, at which time HMR-1 distribution becomes severely disrupted (Fig. 5 A, h). In these embryos, HMR-1 distribution is punctate and discontinuous along cell borders. Double staining for HMR-1 and actin reveals that puncta of HMR-1 correspond to regions where actin bundles remain anchored (Fig. 5 B). Further-

more, areas of punctate HMR-1 distribution were typically found to correlate with areas where CFBs were disrupted (unpublished data), suggesting that the two events are closely linked. Punctate HMR-1 distribution is also observed in the most severe *hmp-1(fe4)* embryos (unpublished data) and in *hmp-1* nulls (Simske et al., 2003). These results indicate that JAC-1 plays a role in maintaining proper cadherin distribution at epidermal cell contacts during elongation, which correlates with maintenance of actin anchorage at adherens junctions.

The research presented here demonstrates that JAC-1, the sole p120ctn family member in *C. elegans*, acts as a modulator of cadherin function during epidermal morphogenesis.

Figure 5. JAC-1 cooperates with HMP-1 to promote the anchorage of CFBs and the maintenance of HMR-1 distribution during elongation of the embryonic epidermis. (A) Images showing representative embryos stained for actin (a–d) or HMR-1 (e–h). (a and e) Wild-type embryos. (b and f) *jac-1(RNAi)* embryos. (c and g) *hmp-1(fe4)* embryos. (d and h) *hmp-1(fe4); jac-1(RNAi)* embryos. In c, the arrow points to an abnormally thick actin filament bundle. In g, the arrow indicates an area with no HMR-1 staining, and the arrowhead indicates an area of punctate HMR-1 staining. All images are lateral views with the dorsal side up. Bar, 10 μ m. (B) An epidermal seam cell from an *hmp-1(fe4); jac-1(RNAi)* embryo double labeled for HMR-1 and actin. Bar, 5 μ m.



JAC-1 cooperates with HMP-1/ α -catenin to both promote the proper attachment of actin filament bundles to adherens junctions and to maintain HMR-1/cadherin distribution in junctions. This, in turn, is necessary for elongation of the embryo into a worm-like shape.

Interestingly, Thoreson et al. (2000) found that p120ctn likely plays a role in mediating linkage between the actin cytoskeleton and adherens junctions during epithelial cell compaction. Cells expressing a p120ctn-uncoupled cadherin mutant had defects in the anchorage of the actin cytoskeleton into E-cadherin plaques, resulting in failed formation of the circumferential actin rings that link epithelial cells into a compact colony (Thoreson et al., 2000). Despite differences in the actin structures involved, both epidermal elongation in *C. elegans* and cell compaction require insertion of bundled actin into sites of clustered cadherin. Our work, and that of Thoreson et al. (2000), suggests that p120ctns play an important role in coordinating organized linkages between the actin cytoskeleton and cadherin-based adhesions. Our studies also point to the utility of *C. elegans* as a model system for exploring how anchorage of the actin cytoskeleton to adherens junctions is regulated.

Materials and methods

Strains and alleles

C. elegans strains were maintained according to standard protocols (Brenner, 1974). The Bristol N2 strain was used as wild type. NL2099 is described in Simmer et al., 2002. *hmr-1(zu389)* is described in Costa et al. (1998).

Determination of *jac-1* cDNA sequences and gene structure

The exon-intron structure of Y105C5B.21 was confirmed by sequencing the cDNA clone yk323d7 (all cDNAs were a gift from Y. Kohara, National Institute of Genetics, Mishima, Japan). To determine the structure of the 5' end of the gene, 5' rapid amplification of cDNA ends (RACE) was performed using mixed-stage *C. elegans* total RNA with the Ambion First Choice™ RLM RACE kit according to the manufacturer's instructions.

Construction of the GFP-tagged *jac-1* transgene and germline transformation

The JAC-1-GFP construct was generated by cloning yk323d7 cDNA into pPD49.78 and replacing the heat shock promoter with the promoter region of pW02-21 (Costa et al., 1998). The GFP coding region from pPD113.54 was cloned into a unique NheI site in the *jac-1* cDNA. pPD vectors were gifts from A. Fire (Carnegie Institute of Washington, Baltimore, MD). Microinjection was performed according to standard protocols (Mello and Fire, 1995).

Yeast two-hybrid assay

The yeast strain PJ69-4A together with plasmids pGBDU-C3 and pGAD-C2/3 (gift from S. Elledge, Baylor College of Medicine, Houston, TX) have been described previously (James et al., 1996). The region encoding the cytoplasmic domain of HMR-1 was cloned into pGBDU-C3. pGBDU-HMR-1 was mutagenized as described in the QuickChange protocol (Stratagene). The region of *jac-1* encoding the Arm repeats and COOH terminus was cloned into pGAD-C3. The coding region of HMP-2 (amino acids 13–679) was cloned into pGAD-C2. The yeast two hybrid assays were performed essentially as described previously (James et al., 1996).

RNAi and RT-PCR

For RNAi via injection, *jac-1* dsRNA was prepared using the cDNA clone yk113h12 and injected at 2 mg/ml into one gonad arm per animal. To score embryonic lethality, animals were injected with *jac-1* dsRNA and cultured together for 6–12 h after injection. The embryos produced by singled worms over the next 36 h were scored for lethality. To obtain embryos for phenotypic analysis, worms were cut transversely through the vulva at 24 h after injection, and extruded embryos were collected.

A *jac-1(RNAi)* feeding vector was created by cloning exons 2–5 of the *jac-1* cDNA into the pPD129.36 feeding vector to generate pPE110. RNAi

by feeding was done as described previously (Kamath et al., 2001). Worms were grown on HT115(DE3) bacteria transformed with either pPE110 or pPD129. Total RNA was isolated from mixed populations of worms using RNeasy isolation kits (QIAGEN). RT-PCR was performed as described in Johnstone (1999). A control RT-PCR product derived from the *ama-1* gene was used to normalize the levels of *jac-1* RT-PCR products between samples. Three RT-PCR reactions were performed for each batch of RNA. RT-PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. Images were captured, and pixel densities for the *jac-1*- and *ama-1*-specific bands were determined using the Bio-Rad Laboratories Quantity One software.

Morphological analysis and embryo staining

4D Nomarski time-lapse video microscopy was performed as described previously (Williams-Masson et al., 1997).

In situ hybridizations were performed using previously described methods (Seydoux and Fire, 1995). Sense and antisense probes were synthesized from the *jac-1* cDNA clone yk533d1. Embryos were mounted and observed using Nomarski microscopy. Images were acquired with a SPOT color CCD camera (Diagnostic Instruments).

For immunostaining, embryos were freeze cracked as described previously (Williams-Masson et al., 1997) and stained as described in Simske et al. (2003). Embryos were stained for HMR-1 using rabbit anti-HMR-1 antiserum (courtesy of J. Priess, Fred Hutchinson Cancer Research Center, Seattle, WA) and a secondary goat anti-rabbit Texas red antibody (Jackson ImmunoResearch Laboratories). Embryos were stained for AJM-1 using a monoclonal antibody (MH27) and a secondary goat anti-mouse Texas red antibody (Jackson ImmunoResearch Laboratories). Phalloidin staining was done as described previously (Costa et al., 1997). For double labeling with HMR-1, 0.2% Triton X-100 was added to the fixative, and embryos were stained for HMR-1 as described above except that Alexa[®]488-phalloidin was added in with the secondary antibody. Staining was observed using laser scanning confocal microscopy. Images were captured using a Bio-Rad Laboratories MRC1024 confocal microscope, and image processing was performed using NIH Image with macros created by J. Hardin.

Online supplemental material

Videos that correspond to Fig. 4 B are available at <http://www.jcb.org/cgi/content/full/jcb.200212136/DC1>.

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